

# Pathology of Stented Common Carotid Aneurysm in Dogs

## Comparison between Stenting and Stent-Assisted Coiling

J.M. LIU, W.Y. ZHAO, X. ZHANG, Y. XU, B. HONG, Q.H. HUANG

Department of Neurosurgery, Changhai Hospital, Second Military Medical University; Shanghai 200433, China

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### Summary

*To elucidate focal successive histological responses of the neck of wide-necked aneurysm after single stent implantation and stent-assisted coiling, an experimental wide-necked aneurysm model was surgically created in bilateral common carotid arteries of adult dogs. Balloon-expandable porous stents were positioned across the aneurysm necks on both sides. The aneurysm cavity of one side was additionally loosely coiled with Guglielmi detachable coils after stent implantation. The dogs were followed up with ultrasonography and angiography, then sacrificed at two days, one month, and one year and the aneurysm specimen was subjected to macro, micro, and electron microscopic observation. Stent implantation and coiling was successful in eight dogs. This investigation observed that single stent implantation slowed down the blood flow within the aneurysm, but barely induced thrombosis within the aneurysm. There was neointima formation over the stent mesh without thrombus within the aneurysm cavity, but it did not cover the whole aneurysm neck at one year. Aneurysms treated with stent-assisted coiling showed thrombosis within the aneurysms and neointima formation over the whole aneurysm orifice. These results suggest that the neointima could develop over bare stent filament without thrombus within the aneurysm cavity, coils could enhance thrombosis within the aneurysm cavity and facilitate*

*neointima formation over the aneurysm orifice. Stent-assisted coiling may be an alternative option for the treatment of wide-necked aneurysms.*

### Introduction

With application of a porous stent in the treatment of intracranial aneurysms, many wide-necked aneurysms can be embolized without clinical complications. However, the detailed focal histological process in the aneurysm neck after single stent implantation or stent-assisted coiling, which has an important influence on strategy decisions in interventional treatment, is still lacking. This study aimed to explore focal histological responses after single stent implantation and stent-assisted coiling to improve strategy decisions in the treatment of wide-necked aneurysms through consecutive observation.

### Methods

#### *Aneurysm Model*

An experimental wide-necked aneurysm model was created by microsurgical suture of a venous pouch to the side wall of the bilateral cervical carotid artery in one surgical session in eight adult mongrel<sup>1</sup> dogs, weighing 15-21 Kg. The dogs received barbiturate anesthesia during operation. The first bolus of heparin 1 mg/kg, followed by 0.5 mg/kg/h was administrated

during the operation. Subcutaneous injection of low molecular heparin (0.2 ml/d) was given for three days after the operation, then oral aspirin of 10 mg/kg.d for six months after surgery. Each aneurysm model was verified by ultrasonography and angiography at 7-28 days to ensure there was no intraluminal thrombosis and the parent artery was patent.

#### *Stent Implantation and Stent-Assisted Coiling*

The dogs underwent interventional treatment when the ultrasonogram and angiogram verified a wide-necked aneurysm had been successfully created without intraluminal thrombus and the parent artery was patent.

A 6F sheath was introduced under general barbiturate anesthesia. A 6F ENVOY guiding catheter was advanced into the carotid artery. The diameter of the balloon-expandable stent (Cordis) was chosen according to the diameter of the parent artery measured in a 3D angiogram. The selected stent was then navigated to cover the aneurysm orifice over a microguidewire. Control angiography was repeated immediately after stent implantation.

After stent implantation in bilateral carotid aneurysms, we randomly selected one aneurysm for stent-assisted coiling. A microcatheter was thus catheterized into the aneurysm through the stent mesh. GDCs (Boston) were detached to loosely pack the aneurysm lumen by 50-60%. All interventional treatments, stenting and coiling, were finished in one session.

The dogs were follow-up by ultrasonography at one and six months, and by angiography at one, three, six and 12 months.

#### *Pathology*

Two dogs were sacrificed two days after interventional treatment, four dogs at one month, and two dogs at one year. The specimen was cut longitudinally for macroscopical observation. The specimens were studied as follows.

One specimen at one month was prepared for microscopic examination after stripping out the stent filaments. The specimen was fixed with 4% polyformaldehyde, dehydrated with alcohol, embedded with wax, sliced in thickness of 5-8  $\mu\text{m}$ , and stained with hematoxylin and eosin (HE). Another specimen at one year was prepared with the stent in situ. The specimen was sliced in thickness of 50  $\mu\text{m}$ . The other processes were similar.

Two dogs at one month and one at one year were subjected to scanning electron microscopy. The specimens were fixed with 4% polyformaldehyde, rinsed three times with 0.1 mol/L phosphoric buffer, fixed again with 1% osmic acid, and coated with gold-palladium. One specimen at one month was subjected to transmission electron microscopy. The specimens were fixed with 4% polyformaldehyde, rinsed three times with 0.1 mol/L phosphoric buffer, fixed again with 7% osmic acid, and stained with citric acid aluminium and uranium acetate.

## **Results**

### *Angiography and Ultrasonography*

Sixteen aneurysms were successfully created in eight dogs. The size of the aneurysms ranged from 4.7x5.0 to 6.3x7.8 mm, and the neck width from 4.5 to 6.5 mm with a neck/body ratio from 0.87 to 1.22.

Ultrasonography showed a non-echo sac (aneurysm) located beside the mid segment of the common carotid artery. Color doppler revealed turbulence within the aneurysm lumen. Axial and longitudinal sections showed a red signal in one half of the aneurysm cavity and blue in another half. The speed of blood flow within the aneurysm was 136-144 cm/s and 145-249 cm/s in the parent carotid artery detected with spectrum Doppler. Angiography verified 16 wide-necked aneurysms. The contrast entered the aneurysm through the distal part of the aneurysm orifice and flowed out through the proximal part with turbulence in the center of the aneurysm. Ten aneurysms showed intraluminal contrast stagnation, but no intraluminal thrombus was found. The parent artery was patent without stenosis.

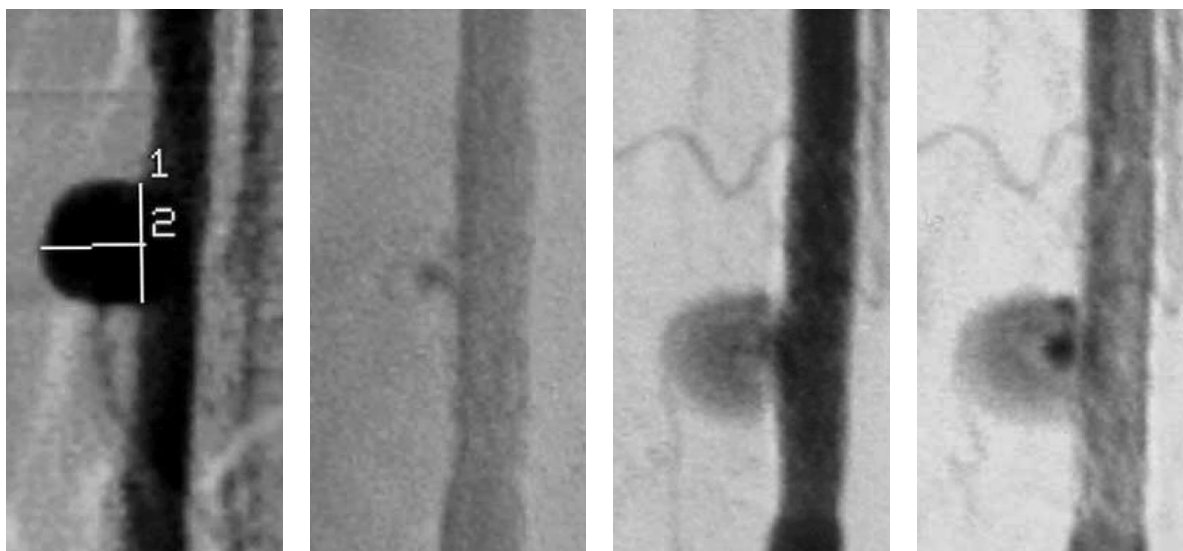
### *Interventional Treatment*

Sixteen stents were implanted across the orifice of aneurysm in eight dogs. Every dog received additional loose GDC coiling in one side after stent implantation. Ten aneurysms showed slowed down inflow, three with reduction in size after single stent implantation.

All eight aneurysms remained partly opacified after stent-assisted coiling. The inflow velocity was slowed down.

### *Follow-up*

Two dogs were sacrificed at two days (see above). Six dogs were examined by ultrasonog-



**Figure 1** Angiography(A-D) demonstrates the narrowed inflow pathway after single stent implantation for wide necked aneurysm. Angiography verified that wide necked aneurysm was successfully created on the side wall of the carotid artery (A). At three months angiographic follow-up, a comma- like inflow was noted on the early phase (B). The contrast slowly filled the aneurysm through a narrowed pathway and the flow was definitely turbulent, but the aneurysm still kept patent (C, D).

raphy at one month. The velocity in the parent arteries of stent-assisted coiled aneurysm was 123-156 cm/s. There was no detectable afferent flow within the stent-assisted coiled aneurysm. The velocity of blood flow was 87-190 cm/s within the parent arteries in the side of single stent implantation and 34-90 cm/s within the aneurysms treated with single stent implantation. Moreover, the flow within these aneurysms became even more chaotic.

Two dogs had ultrasonography at six months. There was still no afferent flow within stent-assisted coiled aneurysms. The velocity in their parent arteries was 78-144 cm/s. The stent implanted aneurysms showed no change in configuration. The afferent flow continued to slow down and ranged 0-30 cm/s, and 82-178 cm/s in their parent arteries.

Six dogs had angiography at one month. No stent showed collapse or displacement. No stent-assisted coiled aneurysm opacified. Three dogs showed even more slowed down inflow within the single stent implanted aneurysms, one with intraluminal contrast filling defect. There was no further change in aneurysm size.

Two dogs had angiography at three months. No stent displaced or collapsed. There was no evident vessel stenosis in the stented segment. The stent-assisted coiled aneurysm still did not opacify. Furthermore, there was a new thin lay-

er forming between the contrast and the stent. The single stent implanted aneurysm showed no change in size, but the afferent flow entered the aneurysm through an evidently narrowed inflow pathway (figure 1). 3D reconstruction showed an irregular shrunken aneurysm neck.

Angiography was repeated in two dogs at six months. The angiogram showed a coil ball shrunk in the stent-assisted coiled aneurysm, and the healing line had become more evident (figure 2). For the single stent implanted aneurysm, the neck continued to shrink but the lumen size remained the same.

Angiography was available in two dogs at one year. No stent displaced or collapsed. The stent-assisted coiled aneurysms were stable. The stent implanted aneurysms remained the same in size, but the afferent flow was very slow with evident stagnation, while the remnant necks became very narrow. Follow-up results by ultrasonography and angiography are summarized in tables 1 and 2.

#### *Pathology of Single Stent Implantation Specimen*

Macroscopic investigation at two days showed no thrombus within the aneurysm lumen. There were no thrombi adhering to the stent, and some filaments of the stent were embedded in the arterial wall.

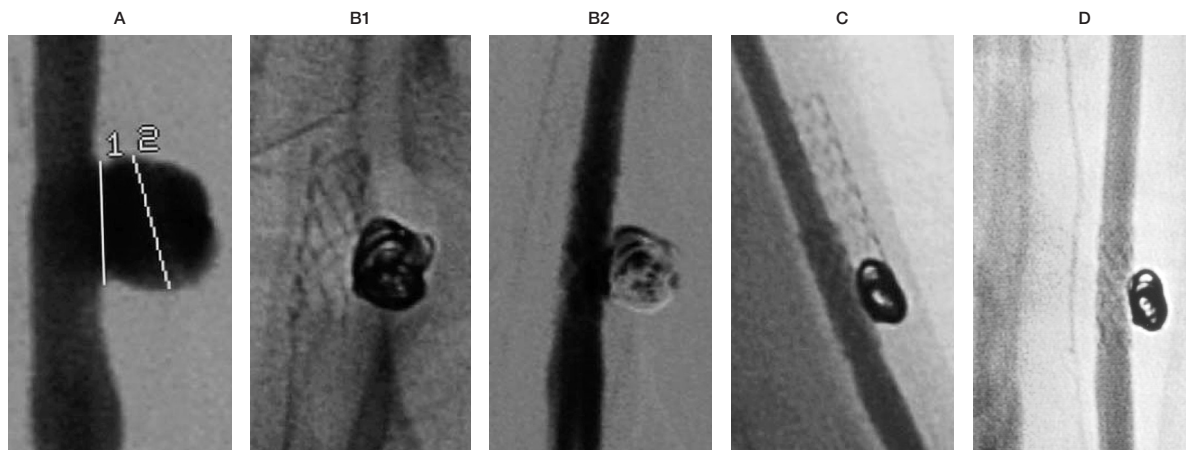


Figure 2 Angiography (A-D) demonstrated the “healing line” in the aneurysm after stent-assisted (bare) coiling for wide necked aneurysm. A wide necked aneurysm (A) was treated with stent implantation and loose coiling (B1). The aneurysm still opacified partially after intervention (B2). Three month later, the aneurysm no longer opacifies and the coil ball shrunk. A thin radiolucent layer between the vessel lumen and the stent emerged (C). At six months, the “healing line” became more evident (D).

Table 1 Ultrasonographic and angiographic follow-up for aneurysms with single stent implantation.

Animal n°	Size	Immed after intervention		At one month		At three months		At six months		Macroscopy at sacrifice
		IFV	config	IFV	config	IFV	config	IFV	config	
1 (sacrificed at 1 year)	5.8x7.6	–	–	↓	–	↓↓↓	NIP	↓↓↓	NIP	No intraluminal thrombus, neck partially covered with neointima but several pores of the stent remained open
2 (sacrificed at 1 year)	6.8x6.5	↓	–	↓	–	↓↓↓	NIP	↓↓↓	NIP	The same as case 1 at 1 year
3 (sacrificed at 2 days)	5.4x5.7	–	–							Some white cotton like tissue adhering to stent wall, which consisted of fibroprotein and scattered lymphocyte verified microscopically. No thrombus adhering to stent or aneurysm wall
4 (sacrificed at 1 month)	6.3x6.5	↓	–	↓	–					No intraluminal thrombus. Most of the stent grids over the neck were covered by neointima formation. The neck was narrowed as several small pores
5 (sacrificed at 1 month)	5.3x6.1	↓	↓	↓↓	↓					The same as case 4
6 (sacrificed at 2 days)	4.7x5.0	–	↓		↓					No intraluminal thrombus within patent aneurysmal lumen
7 (sacrificed at 1 month)	5.5x5.9	↓	–	↓↓	See macroscopy					Intraluminal thrombus, otherwise the same as case 4
8 (sacrificed at 1 month)	7.2x5.4	↓	–	↓↓	–					The same as case 4

Abbreviations:  
 – : no evident change   ↓ : reduced   ↓↓ : further reduced   ↓↓↓ : exceedingly reduced  
 immed: immediately   config: configuration   IFV: inflow velocity   NIP: narrowed inflow pathway

Specimens at one month showed partial thrombosis within the aneurysm lumen in one out of four aneurysms. The segment of the stent overlapping the parent artery was totally covered by a new thin, smooth, glossy and semi-transparent neointima. The neointima seemed a stretch of normal intima and there was no difference between the neointima and normal intima in macroscopy. The aneurysm necks were partially covered by the neointima, with some meshes of the stent over the aneurysm neck left uncovered.

There were no thrombi within two aneurysms at one year. The aneurysm necks were almost covered by neointima except very few meshes still left open. All stent filaments were covered or embedded by neointima formation.

Microscopy showed the inner layers of the artery were endothelial cells grounded by smo-

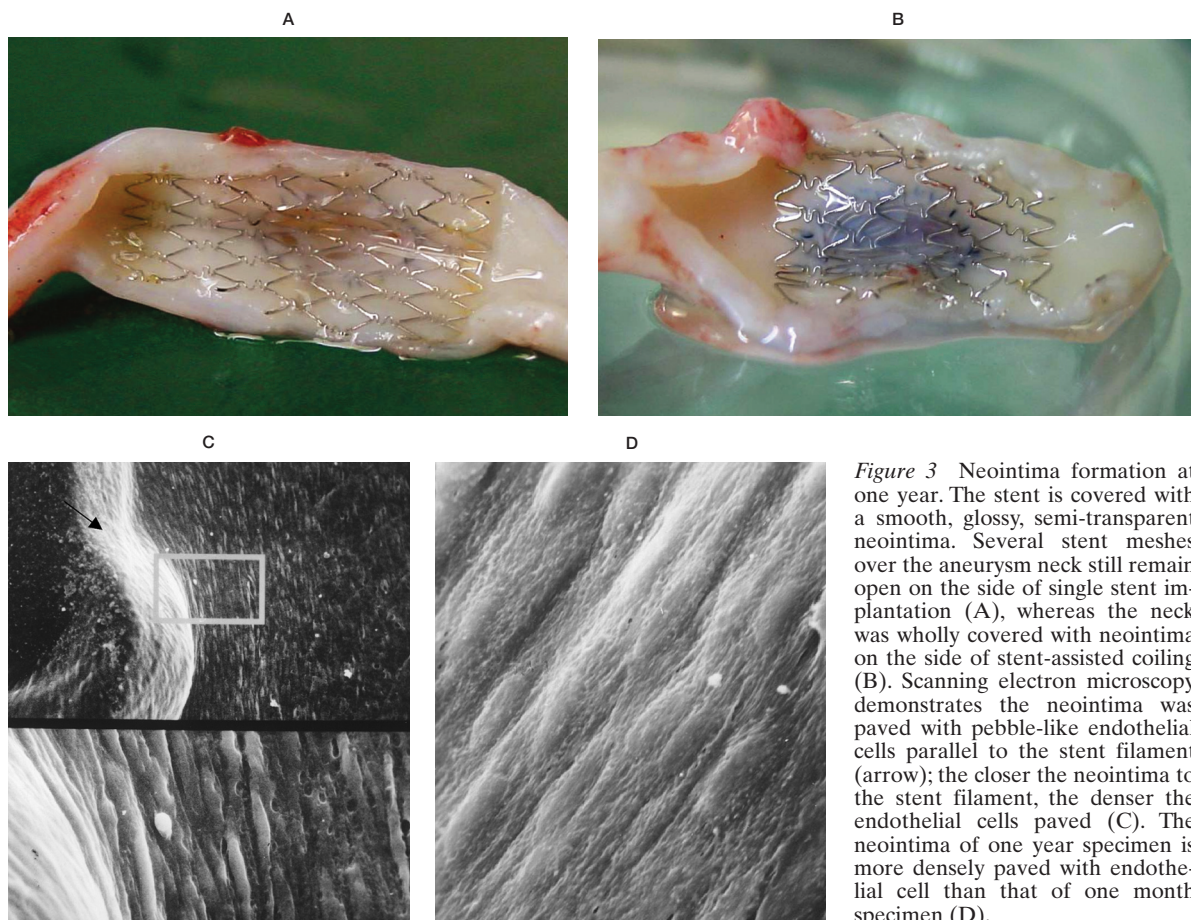
oth muscle and fibroblastic cells and collagen fibers in the stented segment in a specimen at one month. Microscopic examination of one specimen at one year corresponded to the angiographic findings. There was no thrombus or fibrosed thrombus within the aneurysm lumen and most of the orifices were covered by a membrane. Only several large stent meshes kept open. All filaments were surrounded by or embedded in neointima membrane.

The scanning and transmission electron microscopy of a single stent implanted specimen at one month verified some of the stent meshes were covered with neointima. The neointima in the central part of the stent mesh was just collagen, while the neointima near the filament was additionally paved with pebble-like endothelium. The endothelium lining was parallel to the stent filament. The nearer the neointima

Table 2 Ultrasonographic and angiographic follow-up for aneurysms with stent-assisted coiling.

Animal n°	size	Immed after		At 1 month intervention		At 3 months		At 6 months		Macroscopy at sacrifice
		IFV	config	IFV	config	IFV	config	IFV	config	
1 (sacrificed at 1 year)	6.3x7.8	↓	Partially opacified	0	occluded	0	Coil ball shrunken, "healing line" emerged	0	healing line became more evident	The whole stent was covered by thin, smooth, glossy and semitransparent neointima formation at 1 year
2 (sacrificed at 1 year)	5.8x6.5	↓	Partially opacified	0	occluded	0	Same as case1	0	Same as case1	The same as case 1 at 1 year
3 (sacrificed at 2 days)	5.8x5.9	↓	Partially opacified							The aneurysm lumen was full of thrombus. No visible thrombus or tissue adhering to stent wall
4 (sacrificed at 1 month)	6.9x6.3	↓	Partially opacified	0	occluded					Thrombosis within the aneurysm. The neck was covered by neointima except several small pores among the stent and the coil still open.
5 (sacrificed at 1 month)	5.0x5.3	↓	Partially opacified	0	occluded					The same as case 4
6 (sacrificed at 2 days)	5.3x6.2	↓	Partially opacified							The aneurysm lumen was full of thrombus
7 (sacrificed at 1 month)	5.0x6.4	↓	Partially opacified	0	occluded					The same as case4
8 (sacrificed at 1 month)	6.0x6.7	↓	Partially opacified	0	occluded					The same as case 4
Abbreviations: - : no evident change   ↓ : reduced   ↓↓ : further reduced   ↓↓↓ : exceedingly reduced immed: immediately   config: configuration   IFV: inflow velocity   NIP: narrowed inflow pathway										





**Figure 3** Neointima formation at one year. The stent is covered with a smooth, glossy, semi-transparent neointima. Several stent meshes over the aneurysm neck still remain open on the side of single stent implantation (A), whereas the neck was wholly covered with neointima on the side of stent-assisted coiling (B). Scanning electron microscopy demonstrates the neointima was paved with pebble-like endothelial cells parallel to the stent filament (arrow); the closer the neointima to the stent filament, the denser the endothelial cells paved (C). The neointima of one year specimen is more densely paved with endothelial cell than that of one month specimen (D).

was to the filament, the denser the endothelium paving. The aneurysm orifice was mostly covered with neointima in a single stent implanted specimen at one year. The neointima was paved with pebble-like endothelium in the same way as the specimen at one month, but much denser.

#### *Pathology of Stent-Assisted GDC Coiling Specimen*

Macroscopic observation found the aneurysm lumen was filled with thrombus in the two specimens at two days. There was no thrombus adhering to the stent in the portion facing the parent artery. Some filaments plunged into the arterial wall. All four aneurysms at one month showed thrombus formation within the lumen. There was neointima formation over the stent filaments and coils crossing the aneurysm orifice. The orifice was almost covered with neointima. Only several large gaps between the filament and the coil were left open. Specimens at one year showed the stent and the aneurysm

orifice were totally covered with neointima.

Microscopic investigation showed the aneurysm orifice was covered with a membrane and the aneurysm lumen was filled with fibrous tissue at one year. Because of the slice thickness, we could not distinguish their cellular components. But the membrane was subjected to electron microscopic investigation. The results of scanning and transmission electron microscopic investigation of a stent-assisted coiling specimen at one month and one year respectively were similar to those of the single stented specimen (figure 3). However, the aneurysmal lumen with stented-assisted coiling was totally closed by the neointima and the endothelium and denser than in single stent implanted specimen at one year.

#### **Discussion**

Wide-necked aneurysms are always a challenge for treatment whether it is interventional embolization or surgical clipping. Stent im-

plantation for treating intracranial aneurysms is now an option for wide-necked aneurysms<sup>2,3</sup>. As its clinical application is becoming more and more popular, three issues concerning the effects and efficacy of stent implantation in the treatment of intracranial aneurysm are posed.

*1. Can single stent implantation or stent-assisted coiling induce thrombosis within the aneurysm lumen?*

There are always arguments on this issue<sup>4,5,6</sup>. This investigation examined eight aneurysms at different intervals after single stent implantation. Only one aneurysm showed partial thrombosis whereas the others contained no thrombus. For two aneurysms at one year, even though blood flow within the aneurysm was very slow and contrast stagnation was very evident and their necks were almost covered with neointima, there was still no thrombus within the lumen. So we believed single stent implantation could slow down the blood flow within the aneurysm, but the chance of inducing thrombosis after single stent implantation was very low.

There are reports that stent implantation can help prevent the coil from protruding into the parent artery, slowing down the blood flow, and inducing thrombosis<sup>7,8,9</sup>. So stent-assisted coiling is being adopted by more and more interventionists. This investigation treated eight wide-necked aneurysms with this technique. The results suggest that stent plus coiling can induce thrombosis and facilitate aneurysm occlusion. This phenomenon is different from single stent implantation or single coiling, and it seems to us that stent plus coiling could have an overall positive effect. Several explanations are possible. It is logical to think that the blood flow below a certain threshold was the key point for inducing thrombosis when we took into account that both stent implantation and coiling could slow down blood flow within the aneurysm. But this explanation remains incomplete in particular with the two aneurysms with single stent implantation at one year. In these two cases, the blood slowly entered the aneurysm through several small neointima defects on the aneurysm neck and the contrast stagnation was very evident. But there was still no thrombus within the aneurysm lumen. How they have such a strong resistance to thrombosis is still unclear for the authors.

*2. Is there neointima formation over the aneurysm orifice after single stent implantation?*

Some studies indicate stent implantation can facilitate thrombosis and neointima formation<sup>4,5</sup>. But clinical observation<sup>6</sup> and this investigation showed that single porous stent implantation barely results in thrombosis within the aneurysm lumen. To our knowledge, it was still unclear whether there is neointima formation over the stent mesh on the aneurysm neck lacking thrombus within the aneurysm lumen. So doubts have arisen whether the neointima can grow over "bare" stent filament. In this investigation, just one aneurysm showed partial thrombosis among eight treated with single stent implantation. But there was neointima formation over the stent meshes on the aneurysm orifice in all six aneurysms that had been followed up. Furthermore, the neointima continued to spread over the stent mesh on the aneurysm orifice. The aneurysm orifice was almost totally covered by neointima at one year except for neointima defects on several large meshes. The histological examination verified the neointima was paved with endothelium. This result indicates that the stent can act as a scaffold for neointima formation even if intraluminal thrombus is lacking. But the neointima still could not cover the whole aneurysm orifice at one year duration owing to the large gap of the stent mesh suggesting single stent implantation was still not enough to achieve anatomical healing of the aneurysm.

*3. How about neointima formation over the aneurysm orifice after stent-assisted coiling?*

Neointima formation can barely cover a wide-necked aneurysm orifice after mere GDC coiling<sup>10,11</sup>. Stent-assisted GDC coiling may have positive effects on neointima formation over the aneurysm orifice. But objective studies are lacking since the feasible experiment of stent-assisted coiling in 1994<sup>12</sup>. In this investigation, four specimens at one month revealed that most of the aneurysm orifice was covered by neointima formation. The neointima extended alongside the stent filament and the coil over the aneurysm orifice. The main element of the neointima was collagen fiber and the portion near the stent filament or the coil was covered by endothelium. Specimens at one year showed the aneurysm orifice was totally covered by neointima. The endothelium was sustained by collagen fiber, smooth muscular cell, and fibrob-

last cell. The longer the time after intervention, the denser the endothelium paved neointima.

The mechanism of neointima formation over the filament and coil is not clear. It is reasonable to deduce that the stent filament and coil over the aneurysm orifice and the thrombus within the aneurysm lumen could act as a scaffold facilitating neointima development from the arterial wall to the aneurysm orifice.

### Conclusions

In summary, this investigation observed single stent implantation could slow down blood flow within the aneurysm and thus provide pro-

tection for the aneurysm to a certain extent, but could hardly induce thrombosis within aneurysm. Although the neointima could form over the bare stent filament without thrombus within aneurysmal lumen in this group, it did not cover the entire aneurysm neck at one year. Stent-assisted coiling induced thrombosis within the aneurysmal lumen and the neointima paved with endothelium covered the whole aneurysm neck, so the aneurysm achieved anatomical healing. We deduced stent-assisted coiling was an option for treatment of wide-necked aneurysms and densely covering the aneurysm neck was beneficial to thrombosis and neointima formation.

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J.M. Liu, M.D.  
Department of Neurosurgery  
Changhai Hospital  
Second Military Medical University  
Shanghai 200433, China